



Agriculture and  
Agri-Food Canada

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Agroalimentaire Canada

# **ONTARIO RESEARCH ENHANCEMENT PROGRAM (O R E P)**

**Final Report**

## **Sustainable Pollination Services for Ontario's Crops**

**REPORT No. OREP - 1999/19**

**LEAD INVESTIGATOR  
P. G. Kevan**

**Canada**



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**March, 2001**

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## FORWARD

The Ontario Research Enhancement Program (OREP) is a \$4-million two-year federal research initiative that is administered by the Research Branch of Agriculture and Agri-Food Canada (AAFC), with input from the agriculture and agri-food sector, universities and the province. Research focuses on two areas identified by the sector as:

1. Priorities responding to consumer demand for higher quality products; and
2. Ensuring crop-production management systems are environmentally sustainable.

Biotechnology is one of the areas to be explored. Ontario's agricultural production is based primarily on growing diversified crops using intensive production practices, but long-term viability is linked to the development of sustainable crop production management systems.

There are also opportunities to enhance the economic contribution of Ontario's agriculture and agri-food sector by adding value to the diversified primary commodities produced in the province. The Program is expected to be of particular interest to the corn, soybean, greenhouse, fruit and vegetable sectors and the emphasis will be on projects focussing on food quality improvement and sustainable crop production management such as:

1. The development of value-added animal and crop food products and ingredients that meet domestic and export market demands, with a particular emphasis on quality issues such as processing, packaging, shelf life, new product and ingredient development, high-value alternative uses, probiotics and functional foods;
2. Biotechnologies to reduce environmental pressures related to crop protection practices; and,
3. Integrated crop protection and production practices for both field and greenhouse crops.

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## Executive Summary

1. Pollination services are worth about \$500,000,000 annually to agriculture in Canada, with honeybees being the most important pollinators
2. Honeybees are threatened with devastating parasitic mite diseases and already shortages of pollination services have been encountered in the USA and in Canada. Breeding and protection offer solutions, but at a cost to beekeeping and discouragement of beekeepers.
3. Various crops need research to enhance pollination technology, management, reliability, and sustainability to increase efficiency of pollinators used to increase and stabilize harvests.
4. The greenhouse vegetable industry in Ontario is expanding at 20% per annum. Pesticide free crops make this industry highly competitive and bumblebee pollination is part of the growing technology, yet bumblebee services were not adequate during winter and early spring because of the bees' escaping outdoors through vents. UV and colour differences in the plastic greenhouse material compared to the open sky through the vent allow for the bees to orient and escape to the outdoors.
5. Pollination services need to be made more reliable and their diversity increased to assure continued agricultural productivity of insect-pollinated crops.
6. This research breaks into 5 phases, as follows:
  - a. Bumble beekeeping in greenhouses has encountered problems because bees escape through open vents in the winter and early spring and perish in the cold. Research started by monitoring bumblebee activity in relation to temperature, humidity and light regimes in large commercial tomato greenhouses near Leamington. The kind of plastic covering used had a major influence on bumblebee activity and losses. CT proved to be the most "bee-friendly" plastic tested. Applied research based on theory and practice of insect colour vision has been used for considerations of plastic optical and physical barriers over greenhouse vents (Morandin, M.Sc thesis 2000).
  - b. To determine how pollen moves in high density apple orchards, paternal genes from pollen-donors were analyzed from apple seeds. We were able to determine an genetic signature for each of the seven apple cultivars we studied Using that information we found that paternal influence extends

about 9 trees along rows and 13 trees across rows, but most (over 70%) of the influence is within 3 rows. Pollinating bees tend to forage within a few trees, along rows, and either high or low in the trees. Ongoing research seeks to harmonize the movements of genes with the movements of bees with the ultimate aim of determining the pollinator forces needed for pollination of major cultivars of apple in modern high density plantings of orchards (Kron *et al.* in press).

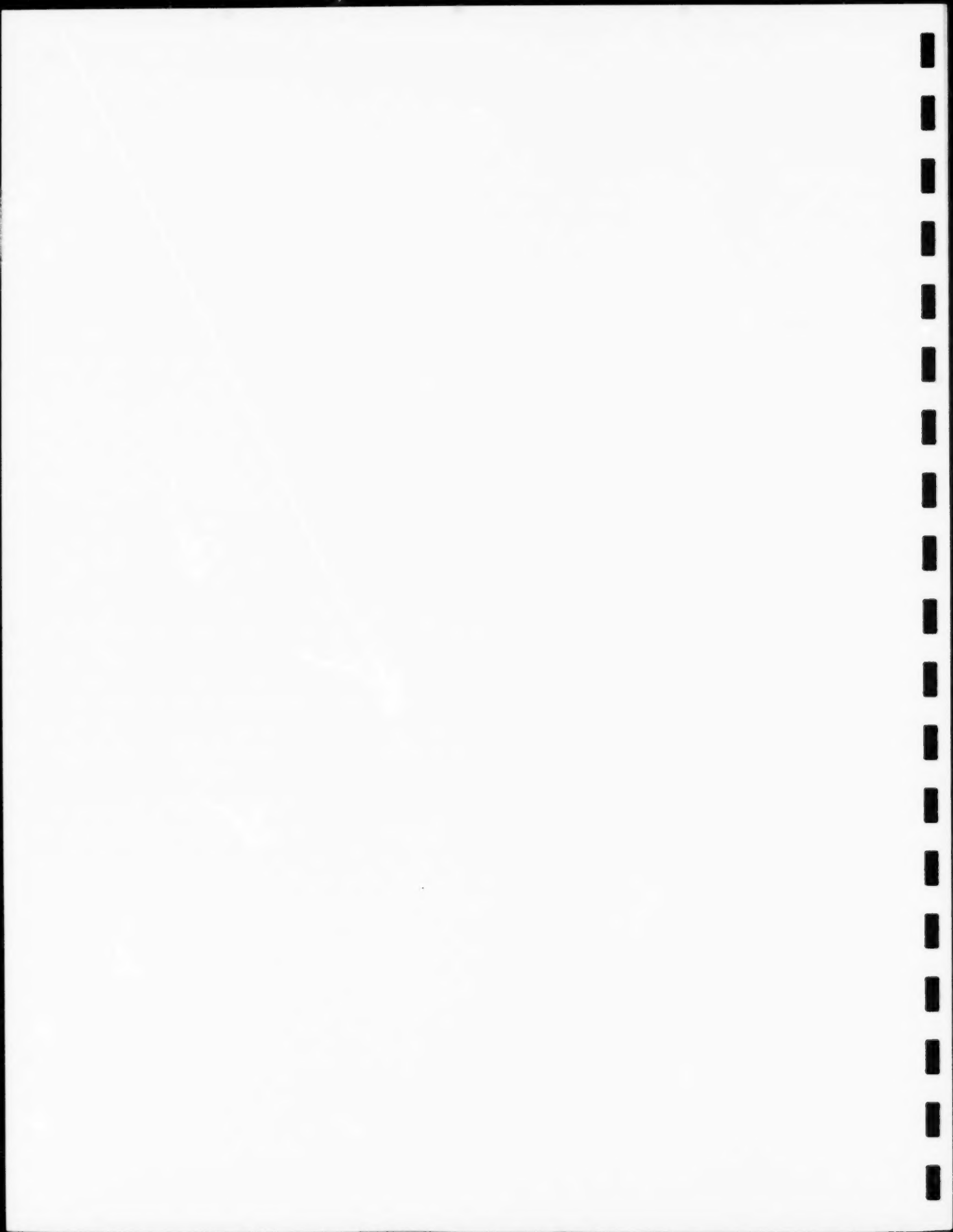
- c. Flowers of different blueberry cultivars have different morphologies, some of which are more conducive to pollination by honeybees. Our preliminary study examined 10 cultivars and we determined which are most likely to be pollinated by honeybees.
  - d. Pickling cucumbers require cross pollination between male and female flowers. Honeybees are largely responsible for field cucumber pollination. On two farms, we found that the intensity of honeybee visitations to cucumber flower declined by 50% over 250 m. On our study fields this was not sufficient a drop-off to adversely affect harvests. Further research has been initiated for continuing this work.
  - e. Research into biology of the hoary squash bee, *Peponapis pruinosa*, a specialist and highly efficient native pollinator of *Cucurbita* crops, showed the bee almost ubiquitous in southern and central Ontario (156 farms surveyed). The bee nests can be protected on fields by medium-, or no-tillage practices.
  - f. Our preliminary study on the comparison of bumble bee, honey bee and native bee pollination of red clover indicates that honey bees are the most effective pollinators of that crop.
7. Readers are cautioned that the work reported herein is preliminary and mostly from the field season of 1998, with a little additional information from the field season of 1999. Full reports on the research from 1998 through 2000 will be placed in the scientific literature and in grower magazines as analyses are completed.

## ANTICIPATED BENEFITS TO THE INDUSTRY

The anticipated benefits to industry are tremendous. The profit levels already enjoyed by growers employing managed pollinators in apple orchards (Kevan 1997), other crops (CAPA 1995), and greenhouse tomatoes in Ontario are already high. With greater reliability of pollination, harvests can be more readily predicted, the crops will be of higher quality, farm gate profits will be higher, competitive ability against imports enhanced, and planning for the future less uncertain.

Specifically, the project will provide the following benefits:

1. to the plastic industry, guides for manufacture of new formulation(s) of plastic film which will allow bumble bee foraging in greenhouses while suppressing grey mould spore germination.
2. allow the greenhouse industry to continue using bumble bee pollination in winter and early spring for tomatoes, green and bell peppers, etc.
3. allow the Ontario greenhouse industry to expand its market competitively into the NE USA with high quality, pesticide free produce.
4. allow the modern apple and orchard industry to more effectively use pollination services provided by beekeepers, and to diversify the pollinator community to increase assurance of greater and higher quality crops.
5. allow the highbush blueberry industry to be more assured of effective pollination by understanding which cultivars are best pollinated by honeybees
6. allow the squash and pumpkin growers greater assurances of effective pollination by encouraging specialized pollinating squash bees.
7. allow greater assurance of pollination of red clover by providing the most effective means of pollination.



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## I. Introduction

Pollination is a highly valuable process to agriculture. For most crops, reliance has been placed with honey beekeeping, or on chance. In Canada, use of honeybees results in \$41 to \$192 per \$1 spent on pollination on some crops (CAPA 1995). Furthermore, in 1996 in Ontario the value of greenhouse crops that require bumble pollination rose to \$112M. Given those figures, it is surprising that more effort is not placed into pollination technology, especially on crops which are poorly understood.

Problems facing crop pollination have been recognized in Canada for many years. At the 1989 Canadian National Workshop on Bee and Pollination Research possible difficulties because of potential declines in the numbers of honey bee colonies in North America were discussed (Agriculture Canada 1989). Since that time most of the predictions for pollination have come true in the USA. Those predictions are held at bay in Canada through vigilance within apiculture and agriculture. However, eventually the problems of tracheal mites and *Varroa* mites which have created havoc with crop pollination services, and caused crop reductions in the USA, have afflicted Canada (Kevan 1990, Grant 1997). Even so, progress in honeybee breeding within Ontario provides some real optimism that the same levels of severe honeybee losses as in the USA will not afflict Canada. Given dwindling numbers of colonies in Canada (CAPA 1995), coupled with the increasing incidence of mite diseases, and the increased cost of maintaining healthy colonies with genetic stock and pesticides, priority must be paid to using honeybees more effectively (Agriculture Canada 1989). It is also important to look towards diversifying the array of pollinating insects that can be used in agriculture. The value of alternative pollinators has been proven for many years through the encouragement of alkali bees, culture of alfalfa leafcutting bees, and more recently through bumblebee (*Bombus*) culture for greenhouse crop pollination (Kevan *et al.* 1992), and orchard bees for orchard fruit pollination (Torchio 1987). Despite those proven successes, much needs to be done to remove pollination services of crops from dependence on single species (e.g. honeybees for many crops, alfalfa leafcutting bees for alfalfa, and *Bombus* in greenhouses).

The rationale of this project is to establish a basis for reliable sustainable pollination services for agriculture in Ontario. Existing technologies must be refined to use available pollinators better (e.g., honeybees on high density apple orchards and bumblebees in tomato greenhouses) and new potentials must be explored to encourage diversification of pollinators (e.g., bumblebees for red clover seed, and

squash bees for pumpkin and squash). The objectives of this proposal are to explore existing technologies to maximize crop production through effective use of pollinators.

The methods used in this project primarily involve investigating bee activity and pollen dispersal within, and between, crop cultivars. Pollen dispersal is an inconspicuous, yet critical process for the reproductive success of wild and domesticated plants. Through the transmission of genes, pollen dispersal influences inbreeding, genetic neighbourhood size and the organization of genetic diversity within and between populations (Wright 1978). Knowledge of pollen dispersal has practical significance, because it is essential for plant breeding and crop/orchard design where the goal is to maximize cross-fertilization among, or self fertilization within, crop genotypes. Despite its evolutionary and ecological significance, reproductive successes *via* pollen have historically been difficult to quantify. Most estimates of reproductive success are based on indirect measures using marked pollen or pollinator observations. Thus, its importance and ecological determinants are poorly understood (Levin and Kerster 1974, Handel 1983).

A more direct method to estimate pollen dispersal is through molecular markers (enzyme electrophoresis) (Ellstrand 1984). This approach has been used to estimate variation among plants in reproductive success *via* pollen (Meagher 1986) as well as pollen dispersal distances both within (Campbell and Waser 1989) and between populations (Wyatt and Broyles 1994). Usually, research on pollen dispersal using genetic markers has focussed on the magnitude of pollen-mediated gene flow and the distribution of dispersal distances because of their impact on the scale and rate of evolutionary divergence via drift or selection (Kron *et al.* in press). However, few studies have undertaken a more comprehensive analysis of the ecological and genetic features of pollen donor and recipients that may contribute to variation in male reproductive success. The portions of this project that focus on such an analysis involve high density apple orchards and field cucumbers.

To allow most efficient use of the season, this project focused on a few, model crops which bloom asynchronously in the following order: greenhouse tomatoes, apples, blueberries, field cucumbers, red clover, and pumpkin and squash. The primary questions that need to be addressed for these crops do not all involve the genetic and ecological factors which contribute to reproductive success. Other portions of the project examine particular issues regarding the sustainable pollination of Ontario's crops. For greenhouse tomato production the problems do not lie within

the movement of pollen through the crop, but rather with bumble bee behaviour under different greenhouse conditions. With red clover the primary question of interest for growers is whether bumble bees or honey bees are more efficient pollinators. The problem with blueberry pollination by honeybees is that the floral morphology of some blueberry cultivars is not suitable for honeybee pollination.

The crops and the pollination problems that have been examined are discussed below in greater detail.

### **I. 1. Greenhouse tomatoes and pollination by bumblebees**

The use of bumble bees to pollinate greenhouse crops has become increasingly common. Traditionally, pollination was accomplished with hand held electronic vibrators. Because of expense, risk of damage to the flowers and developing fruit, and the time-consuming nature of this method, there has been interest in developing alternative methods of pollination. The value of greenhouse tomato crops in southern Ontario was estimated at \$78 million in 1996. Before 1992, nearly all greenhouse tomato crop pollination in Ontario was done by hand vibration. As of 1994, all commercial growers in southern Ontario began to use bumble bees as their sole means of pollination, creating a new multimillion dollar supply business (Figure 1).

Bumble bee keeping in greenhouses has encountered some problems because of the escape of the bees (value ca. \$10 - \$20 per bee) through open vents in late winter and early spring. The bees freeze outside and are lost to the colonies (Khosla and Shipp pers. comm.). New understandings in insect colour vision (Backhaus 1993) have suggested ways by which the visual target of the open vents (daylight with UV) (Figure 2) *versus* the greenhouse roof and walls (attenuated UV) can be disguised by coloured transparent flaps or blocked by screening so that the bees are not attracted and can not escape. The former would be easier and cheaper. UV light encourages the germination of spores of grey mould (*Botrytis*), so UV opaque plastics are favoured. Thus, trials are needed to determine the minimum UV transmission to permit bumblebee activity in the greenhouse, with mechanisms to prevent their escape, while suppressing germination of grey mould spores.



**Figure 1.** Bumblebee colonies in place for pollination of greenhouse tomatoes in Essex County, Ontario.



**Figure 2.** The open vents of a typical tomato greenhouse can be seen as brighter than the daylight being transmitted by the plastic covering. To the bees' eye, if the ultraviolet part of the daylight spectrum is strongly depleted by the plastic, the colour of light from the open vent would be different (white, by analogy to human colour vision) from that coming through the plastic (yellow, by analogy to human colour vision). Ultraviolet light is used by many insects in their photopositive escape responses. Greenhouse from Essex County, Ontario.

Determining the activity patterns of bumblebees under various conditions of temperature, humidity, and light in greenhouses made with different plastic coverings is important to understanding how to best manage pollination. This information will ultimately be used to create a standard bumble bee pollination methodology, based on individual greenhouse specifications, for greenhouse tomatoes that can be used by growers and bee suppliers as a procedural guide.

## **1. 2. High density apple orchards and pollination by honeybees**

Although much is known about apple pollination (Free 1993) and the value of honeybees and mason bees for apple production (Torchio 1987), little information is available on the size of the pollinator forces (Kevan *et al.* 1986) required to effect economically profitable levels of pollination in modern orchards with high density plantings using dwarfing root-stocks. The tendency of honeybees to work the trees by row suggests that paternal (i.e., pollenizer) plantings are different from those recommended in standard orchards, but detailed recommendations need further research. The aim of this project is to investigate the mechanisms of pollination in high density apple orchards. It is reasoned that with the decline in both the availability of honeybees in feral colonies and through the reduction in hobby beekeepers, as well as the general chronic shortage of native pollinators, a pollinator crisis could jeopardize apple production. We investigated how bees move in high density apple orchards, within and between trees in rows, and across rows and coupled those findings with the actual movement of pollen as measured through movement of paternal genes as they occur in seeds.

Pollen dispersal and siring success have special significance in commercial apple orchards. Apples are self-incompatible, and therefore fruit production occurs only with pollen transfer between cultivars (Free 1993). To complicate matter, most orchards consist of a limited number of cultivars which are arranged in monotypic blocks or rows. The potential for pollen wastage by pollination between trees of the same cultivar and the resulting economic costs because of lost yields, highlights the need to predict patterns of pollen movement in orchards accurately. Pollen dispersal in apples has received considerable attention; however most studies are based on apparent rather than realized pollen dispersal (e.g., Wertheim 1991) and there have been few attempts to relate factors other than distance to pollination success. Furthermore, few studies have been conducted on more contemporary, high-density orchards. On these orchards, apples are grown on dwarf rootstock for early yields and low shading, but

the rows are planted at such high densities that pollinators may be more likely to move along rather than across, which is necessary for successful pollination (Dale *et al.* 1992).

We examined bee movement on flowers, and within and between trees as well as gene flow and male reproductive success in two apple orchards. For the latter portion of the work we focussed on a single apple genotype, whose paternity could be tracked unambiguously in all cultivars within a large block of each orchard. Using paternity exclusion analysis of seeds, we estimated its siring success on trees throughout the orchard.

### 1. 3. Field cucumbers and honeybee pollination

Honeybees are the pollinators of choice for field cucumber growers, and especially for machine harvested fields because colonies can be moved quickly into fields at the appropriate time when bloom commences. Although field cucumbers do not set fruit without pollination and inadequate pollination results in deformed fruit (Figure 3), in Ontario there are growers that do not bring honeybees to their fields.

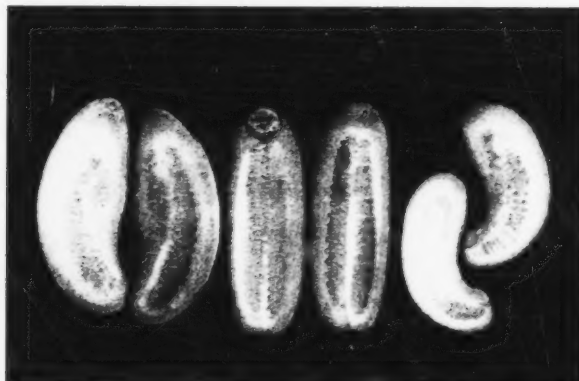


**Figure 3.** Picking cucumbers from a field near Simcoe, Ontario. In the centre are two halves of a well-formed and well-pollinated cucumber. To either side are crooked cucumbers with fewer well developed seeds along one side, the results of inadequate pollination. Crooked cucumbers are less valuable in processing.

Issues associated with the increasing importance of machine harvesting pickling cucumbers include a narrower temporal window for flowering and pollination to make for greater uniformity of cucumber size at harvest time, and associated with that the constraints on the time and intensity of pollination.

It has been found that inter-plant cross-pollination maximizes crop yields. Through isozyme analysis, the pollenizer (pollen-donor) cultivar's genes present in the seeds can be identified in crops. These techniques have been used to examine the movement of pollen in agricultural crops, such as apples (Kron *et al.*, 2001) and mangoes (Dag *et al.*, 1998). As with the apple pollination portion of the project (see above), we incorporated bee behaviour observations with genetic finger printing to gain an understanding of pollen movement through cucumber fields.

#### **I. 4. Pollination problems in highbush blueberries**

Highbush blueberries are not well understood from the viewpoint of their breeding system (incompatibility is used to refer to intervarietal and intravarietal incompatibility, and to self-incompatibility proper) (Eck and Childers 1966). The cultivars grown in Ontario are no exception. Further, the value of honeybees in pollination of highbush blueberries varies from low to nil with cultivar and floral structure. In this preliminary study, floral morphology of 10 commonly grown blueberry cultivars was measured to provide an estimate for which cultivar honeybees would most effectively pollinate. Flowers of highbush blueberry cultivars are variable in corolla depth and aperture width. Thus, some long-tongued bees, especially bumblebees, are more efficient pollinators than honeybees. Honeybee tongues are only about 6mm long and they cannot obtain nectar from some cultivars.

#### **I. 5. Hoary squash bee**

Squash and pumpkin are pollinated in southern Ontario by a specialist bee, the hoary squash bee (*Peponapis pruinosa*) (Figure 4). The females, which are solitary ground-nesters, emerge in summer coincidentally as the crops bloom and they forage on the flowers. The bees become active at dawn and have completed their floral visitations for the day by noon. This time corresponds with the opening hours of the flowers, which last only for one day. This species is entirely dependent on pumpkin and squash for its existence: it does not forage at flowers of any other plants.



**Figure 4.** A hoary squash bee (*Peponapis pruinosa*) in a pumpkin flower near Cambridge, Ontario.

Morse (Cornell University, personal communication) has suggested that in New York State, this bee may be responsible for about 90% of squash and pumpkin pollination. These bees lend themselves to consideration as pollinators for pumpkin and squash growing operations (Willis and Kevan 1995). It should be possible to establish nesting beds, similar to those used for alkali bees, once more is known about the bees' requirements for soil texture, moisture, pH, and so on (see Kevan *et al.* 1988). To this end, a survey of 156 farms throughout southern and central Ontario was conducted.

#### **I. 6. Red clover pollination**

Bumblebees have often been noted as efficient pollinators of red clovers (Free 1993). Honeybees have also been used as pollinators, but their efficiency has been questioned (Free 1993), especially regarding red clover. On red clover, lesser efficiency has been attributed to the length of the corolla tube in relation to the length of the bee's tongue and the inability of the honeybees to obtain the deeply hidden nectar (Free 1993). In our study we attempted to determine the relative efficiency of bumblebees, honeybees and native bees under field conditions.

## II. Objectives

1. to provide guidelines for the manufacture of new formulation(s) of plastic film which will allow bumble bee foraging in greenhouses while suppressing grey mould spore germination.
2. to improve bumblebee pollination of greenhouse tomatoes in double polyethylene greenhouses during the winter and early spring months when the bees often fly out through vents and perish in the cold.
3. to determine the pollinator forces needed for pollination of the major cultivars of apple in modern high density orchards.
4. to assess the most suitable pollen donor cultivars for each apple cultivar.
5. to determine how pollen moves in high density apple orchards so as to provide recommendations on varietal planting patterns to optimize high quality apple yields.
6. to survey flowers of highbush blueberry cultivars in order to determine, based on floral morphology, which cultivars are best suited for honey bee pollination.
7. to determine the nature of honeybee pollination in field cucumber production.
8. to determine where in Ontario the hoary squash bee can be found.
9. to determine the architecture of the subterranean nests of the hoary squash bee in order to assess which tillage practice (high, medium, no) are least likely to destroy the nests .
10. to compare the efficiency of bumble bees, honey bees and native bees for red clover seed set.

### III. Materials and Methods

#### III. 1. Greenhouse tomatoes and pollination by bumblebees

For the bumble bee pollination portion of the project we: 1) examined the optical properties of commonly used greenhouse plastics; 2) studied bee activity under those plastics under commercial greenhouse conditions; and 3) examined bee loss through the ventilation systems of greenhouses with different plastics. This work took place in 12 commercial greenhouses in Leamington, Ontario. All of the greenhouses were primarily devoted to growing tomatoes and used 'gutter venting'. Gutter vents are hinged openings that extend the length of the roof of the greenhouse. In all of the greenhouses used in this study, the vents were not screened, and as a result bees were able to exit the greenhouse when the vents were open. Beefsteak tomatoes (*Lycopersicon esculentum* Mill. Solanaceae) were used in the greenhouses, with Trust and Grace being the most common cultivars. The bumblebee species *Bombus impatiens* was used exclusively in all greenhouses. Only greenhouses that were greater than 10 acres were used, and with the exception of one greenhouse, all used only one plastic type. In the case of the exceptional greenhouse, AT Bee Poly plastic and Dura 3 plastic were used in the same greenhouse. However, the monitored colonies for the two different plastic types were separated by 200m. For more specific details on the greenhouses used in this study see Morandin (2000).

##### III. 1.1. Optical properties of greenhouse plastics

The plastic sheeting and screening used in commercial greenhouse construction was typified by spectrophotometry (transmission and reflectance) as provided by the manufacturer (transmission) and as measured (transmission and reflectance) by ourselves. Our typification of the plastics involved making small, double-walled, tents and spectrally measuring the photic environment within, and outside in natural daylight, the tents. We used a portable spectrophotometer to measure the wavelengths of light passing through five types of commercial greenhouse plastics (AT Bee Poly (AT Plastics, Ontario), De Klerk (Klerk's Group Plastic Products Mfg., Inc), CT (Huntsman Corp., USA), Patilux (Pati Corp., Armin Films, USA) and Duratherm 3 (AT Plastics)) over the course of a day. We created a tent of two open-ended bags (ca. 50 cm x 50 cm x 75 cm tall) from each plastic. One bag was placed inside the other in order to simulate the double thickness of plastic that these types of greenhouses use. The bags were placed over a wire frame and the sensor from the

spectrophotometer was inserted inside. These tents allowed us to get an accurate reading of the light passing through the plastics. We also obtained wavelength data for unobstructed sunlight. With this information we were able to make a comparison between the two data sets, unobstructed sunlight and the transmission wavelengths. The procedure was carried out over the course of a day so that we would be able to determine if a change occurred across time. Once the data were collected and transformed into an Excel spreadsheet, the data from the plastic transmissivities were subtracted from the unobstructed sunlight to determine the wavelengths which were able to pass through the plastic.

### III. 1.2. Bee activity

The bee activity experiment was carried out from April 28 to May 26 1999. We grouped the greenhouses into four categories based on plastic types: 1, CT; 2, AT Duratherm; 3, Patilux; and 4, AT Bee Poly and De Klerk. The two plastic types in category 4 were grouped together because they had similar light transmission (see below). We investigated bee activity based on greenhouse plastic type, as well as light intensity and greenhouse temperature. With assistance from technical personnel at U of Waterloo, bee counters were developed for monitoring of bee activity (see Figure 5 and Appendix 1).

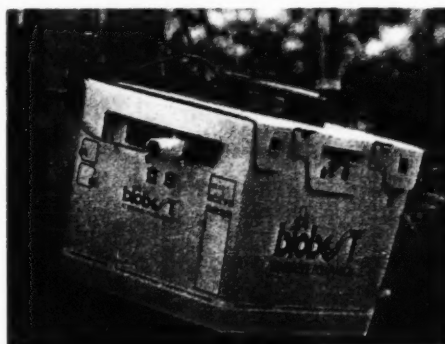


**Figure 5.** A bumblebee hive equipped with an activity monitor (grey entrance tube) as developed as part of this project (see also Appendix I) and as used in tomato greenhouses in Essex County, Ontario.

With those counters data were collected on bee activity and bee loss under the 4 different greenhouse plastic types. Three sets of four greenhouses, one from each of the plastic groups, were monitored for four days each. Data collection in the three sets of four greenhouses was repeated three times, for a total of 36 activity readings for each plastic group. At the start of the first round of data collection, five unopened colonies were set up in each of the 4 greenhouses. The number of bumblebees per colony was recorded prior to opening the colony. All 20 colonies were fitted with a bee counter and opened on the same day. After sunset, on the evening prior to the data collection day, numbers of bees per colony were recorded and the bee counters were set to zero. The following evening, after sunset, counter numbers were recorded.

Repeated measures analysis was used to determine if activity was different among the 4 plastic types, followed by Scheffe's pairwise comparisons. Interactions between day, plastic and round of collection were assessed. In all experiments, significant differences amongst means were detected using  $\alpha = 0.05$ .

### **III. 1.3. Bee loss**

The actual number of bees lost through the ventilation systems could not be assessed directly, hence, colony size was used as an indicator of the number of bees lost. Data were collected for the bee loss experiment from March 7 to 31, 1998. The number of bees in each colony was estimated by a visual count prior to the colonies being opened. Colony size was then monitored in conjunction with the activity experiment. Seven bee counts were used for two sets of greenhouses. The mean proportion of bees per colony was transformed (arcsine square root) and repeated measures analysis was used to test for differences between means based on plastic type. Scheffe's pairwise comparison was used to test for differences between plastics. In-hive mortality of the bees did not seem to be a factor.

### **III. 2. High density apple orchards and pollination by honeybees**

This portion of the project took place in two commercial apple orchards (Farmer Jack's and DwarfTree) near London, Ontario in early May 1998, and 1999. In both orchards, trees were organized into rows with a road running perpendicular to the rows through the centre of the orchard. Honeybee colonies were placed approximately every 11 rows along the main road during the bloom period.

There are 4 parts to the apple experiments:

- 1) observations of bee movements through trees and rows;
- 2) phenology of apple bloom;
- 3) hand pollinations of Ida Red flowers with other cultivars and
- 4) genetic finger printing to determine how pollen is dispersed throughout apple orchards.

The main cultivar we investigated was Ida Red because we were able to find genetic markers for it.

### **III. 2.1. Bee activity**

To understand how pollinating honey bees move on flowers and between trees in high density orchards, honeybees visiting apple tree blossoms and their movements were observed. Bees were observed as they foraged on Ida Red, Jonagold, and Red Delicious. We used the latter 2 cultivars for comparison with observations on Ida Red, however, for this study, we concentrated on Ida Red. Observations were made at various distances away (10m, 25m, 50m, and 70m) from the bee hives to determine if distance has an influence on bee behaviour. As closely as possible, observations were made throughout the bloom period for each of the 3 cultivars. The observations which were recorded include if the observed bee was pollen or nectar foraging, if the bee was foraging on the king blossom, foraging time, bee position (Top = when the bee is positioned on top of the anthers and is foraging for pollen; Side = when the bee is on the petals and is foraging for nectar; In = when the bee is on top of the anthers and is foraging for nectar; and Out = when the bee is on top of the anthers and is foraging for nectar), and bee movement (up, down, away from trunk, to trunk).

To help reveal how bees forage within and between trees, maps of bee movement were made of individual bees as they foraged. This was done only on Ida Red. Poor weather conditions and resulting lack of bee activity limited the depth of this study.

### **III. 2.2. Phenology**

Floral phenology data were collected for 14 cultivars (Vista Bella, Russett, Paula Red, Ida Red, MacIntosh, Spartan, Cortland, Empire, Mutsu, Jonagold, Red Delicious, Granny Smith, Gala and Northern Spy). These cultivars were chosen because they occur within the orchards and may compete with Ida Red for pollination opportunities.

For each cultivar, 10 trees were marked at regular intervals along the entire length of a randomly selected row. On each tree 3 branches were randomly selected. The trees were checked every second day and the total number of open flowers recorded. Counting ended when the number of open flowers on all cultivars had dropped below 10% of the cultivar maximum.

After a period of intensive observations and data collection during pollination (usually May), the apple studies recommenced at harvest time. Individual apples (from inflorescences and trees individually marked in spring during bloom) were collected and assessed for, weight, form, seediness (Kron *et al.* in press).

### III. 2.3. Hand pollination

Ida Red trees were crossed with trees of five cultivars: MacIntosh, Mutsu, Red Delicious, Empire and Northern Spy. One branch was selected from each tree and marked below the fifth cluster of blossoms. The branch was then covered with a pollination bag to prevent bee visitation. Flowers on four of the clusters were pollinated with Ida Red pollen, and the fifth cluster was self-pollinated by hand. In August fruit set was calculated and seed production values calculated.

### III. 2.4. Genetic fingerprinting

Through a preliminary isozyme screen we identified Ida Red as a genotype (cultivar) that could readily be identified in a paternity analysis of seeds. An isolated row of Ida Red trees was then located in each orchard. On August 27, 1998, ripe apples were collected from trees in the Ida Red row and from 9 neighbouring rows (containing MacIntosh, Mutsu, Red Delicious, Northern Spy and Empire cultivars). These 9 rows were spaced 1, 2, 3, 5, 7, 9, 12, 15, and 18 rows in one direction away from the Ida Reds. Three trees were sampled from each row and a total of 12 apples were selected from each tree. Apples were refrigerated intact until seeds were required for electrophoresis.

At least 30 seeds from each tree were used for gel electrophoresis. Gels were stained for 6 isoenzymes: 6-phosphogluconate dehydrogenase (6PGD), aspartate aminotransferase (AAT), acid phosphatase ( $\alpha$ ACP), alcohol dehydrogenase (ADH), phosphoglucose isomerase (PGI), and phosphoglucomutase (PGM). The Ida Red genotype was unique among the cultivars in the 2 orchards, thus the percentage of

seeds sired by Ida Red for each of the 27 maternal trees sampled in each orchard could be determined.

### **III. 3. Field cucumbers and honeybee pollination**

Field observations were made at two farms located in Simcoe, Ontario. The field used in 1998 on first farm had fourteen honeybee colonies that were clumped together at one corner of the field. The cultivars which were studied are FanciPak, FanciPak-M, Score and Vlasset. In 1999 the hives were again clumped together, however, the hives were separated from the cucumber field by a pond and a row of trees. The cultivars studied were studied Patton and Vlasset. The field used in 1998 on the second farm had fourteen honeybee colonies placed along the field-edge in groups of two. The field used in 1999 was similar to the field in 1998. The cultivars which were studied are Patton and Vlasset.

#### **III. 3.1. Bee activity**

For each day that pollinator movements were observed, 100 flowers on different fruit bearing plants were examined to determine their sex ratio. During 1998 and 1999 we studied bee movements on cucumber fields located at the two farms. While the honeybees were foraging on cucumber flowers the number of bee visits during 15 minute intervals were observed at 25 metre intervals from the edge of the field closest to the bee hives. The sex of the flower which was visited was noted.

#### **III. 3.2. Genetic fingerprinting**

During the 1999 growing season, our research took the first step towards using electrophoresis. We acquired seed from Bicks' Pickles which came from three suppliers (Sunseed, Asgrow, Harris Moran) as well as from ripe cucumbers collected at the two farms used for the pollinator movement portion of the study. We also conducted an intensive search of the scientific literature to determine which enzymes were likely to be useful for distinguishing different cultivars. Once individual cultivars could be electrophoretically identified, it was possible to address the question of what extent fruit-bearing cultivars are being pollinated by male flowers on the same cultivar as opposed to pollenizer cultivars. This form of pollination, which is effectively self-pollination, may be occurring frequently and resulting in fruit that are smaller and lower quality.

We collected Patton and Vlasset cucumbers. For Patton we assayed 5 seeds per fruit for 22 fruit, and 6 seeds for 1 fruit for a total of 116 seeds. For Vlasset, we assayed 5 seeds for 15 fruit, 4 seeds for 1 fruit and 1 seed from 3 fruits for a total of 82 seeds.

### **III. 4. Pollination problems in highbush blueberries**

Twenty flowers each from 10 cultivars (Berkeley, BlueCrop, BlueJay, BlueRay, Duke, Elliot, Jersey, NorthBlue, NorthLand, and Patriot) grown in Ontario were obtained. Flower lengths and widths of their openings were measured. The measurements were analyzed with ANOVA and Tukey's test was used to determine which cultivars differed from one another.

### **III. 5. Hoary squash bee**

We conducted a preliminary survey to determine the range of *Peponapis pruinosa* in Ontario. For this survey we recorded only the presence or absence of the bees from fields or gardens which were growing squash or pumpkin. We examined a maximum of 100 flowers in each field or garden for the presence of the bees. As well, in order to determine some of the nesting requirements of the bees, we made resin casts of 15 *Peponapis* nests in Mississauga, Ontario. For excavating the casts we followed the method described in Chapman *et al.* (1990).

### **III. 6. Red clover pollination**

The farm used for this privately funded portion of the project was located near Caledonia in southern Ontario. The growers who funded this work have requested a temporary hold on the release of the results. Colonies of honeybees and of bumblebees were placed, well separated from each other, along one edge of a long field grown for red clover seed production. Study plots were monitored for bee activity throughout the bloom. Harvests of seeds were made from those same plots and the growers used GPS and yield recordings to provide a map of the field showing yield variability.

## IV. Results & Discussion

### IV. 1. Greenhouse tomatoes and pollination by bumblebees

Greenhouse plastics effect tomato pollination by influencing bee activity and bee loss through the ventilation systems. There is a significant difference in bee loss among plastic types ( $F_{3,4} = 6.29$ ,  $p = 0.010$ ). CT plastic, one of the less commonly used greenhouse plastics, experienced less bee loss than AT Duratherm, Patilux, and both AT Bee Poly and De Klerk together ( $p = 0.004$ ,  $p = 0.004$ ,  $p = 0.010$  respectively). The likely explanation for these results is that under CT plastic, bees are less able to visually detect the open vents because of the relatively small contrast between the transmitted light and the light through the vents. Our spectrophotometry results (Figure 6) indicate that of the plastics tested, only CT plastic allows transmission of UV light. Because of this, fewer bees fly out of these greenhouses and die during the winter months. Not only is there less bee loss in CT greenhouses, bee activity was 129.3% higher in those greenhouses than the others which were tested ( $F_{1,28} = 14.922$ ,  $p = 0.001$ ) (Figure 7). Higher bee activity leads to more and better pollination (Figures 8 and 9) of tomatoes and results in higher quality fruit.

For the duration of these experiments the average daily temperature in the CT greenhouse was 6.3% higher than the Patilux greenhouse ( $F_{1,28} = 6.79$ ,  $p = 0.015$ ). Although higher internal temperatures in the CT greenhouse may have been partially responsible for the greater activity observed, it is unlikely that the 6% higher temperature in the CT greenhouse could have been solely responsible for the 4-fold increase in activity. The higher temperature, along with the greater amounts of UV light, may both have been factors resulting in the greater activity observed in that greenhouse.

Maximizing bee activity and minimizing bee loss have important economic consequences on bumble bee greenhouse pollination. Currently, bumble bee supply companies are mostly servicing tomato growers on a supply basis. Greenhouses that experience large amounts of bee loss and/or low bee activity require many more colonies than was originally estimated. Consequently, bee suppliers are losing money in some situations, and have changed their billing to a fee per colony basis. For many growers this has resulted in increases in the price for crop pollination.

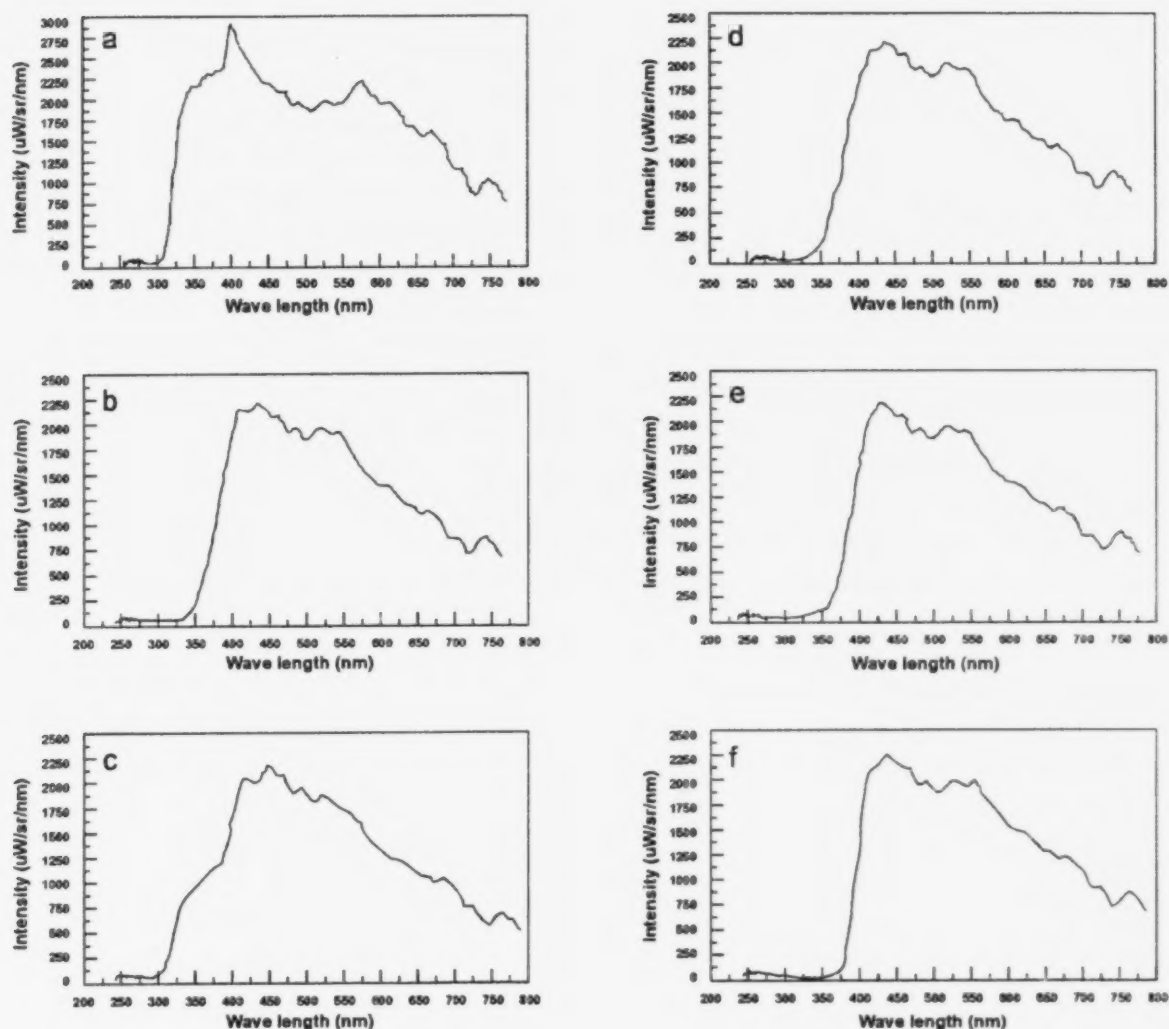


**Figure 6.** Spectral distributions of daylight (a) and light as transmitted through various plastics used for tomato greenhouse coverings in Ontario: (b) is AT Bee Poly plastic; (c) is CT plastic; (d) is De Klerk plastic; (e) is Patilux plastic; and (f) is Dura 3 plastic. Measurements made as part of this study.

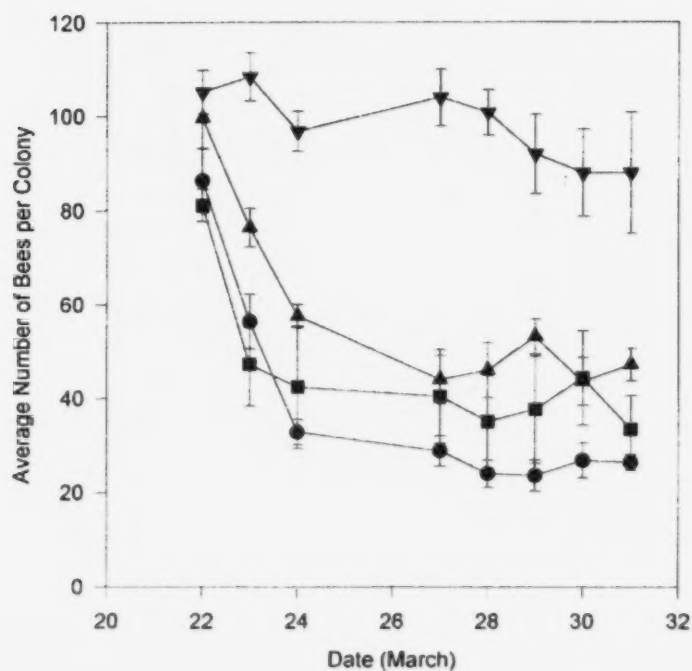
**Bee Loss (March 22-31)**

**Figure 7.** An example of bee losses (March 22 - 31, 1998) from tomato greenhouses covered with various plastics from the study of Morandin (2000) conducted in Essex County, Ontario. CT plastic is associated with statistically significantly the least losses.

Pollination Levels Determined by the Degree of  
Necrotic Discolouration on the Anther Cone

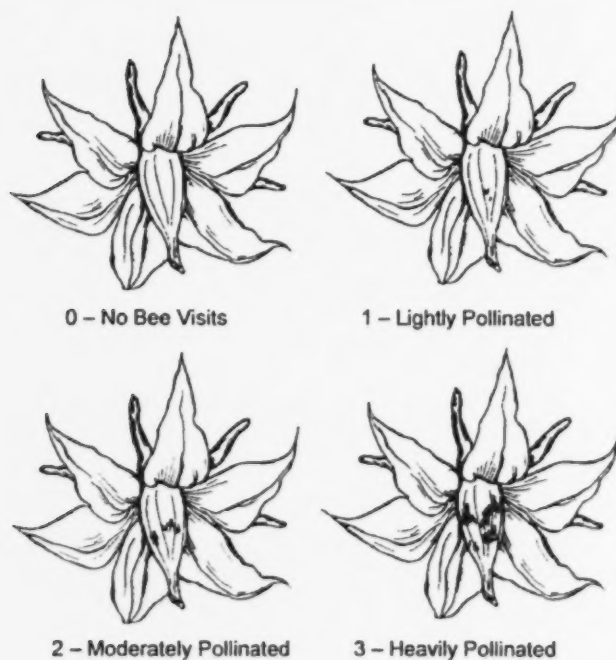


**Figure 8.** Pollination levels as determined by the degree of necrotic discolouration (bruising) of the anther cone caused by the bites of pollinating bumblebees (from Morandin 2000).

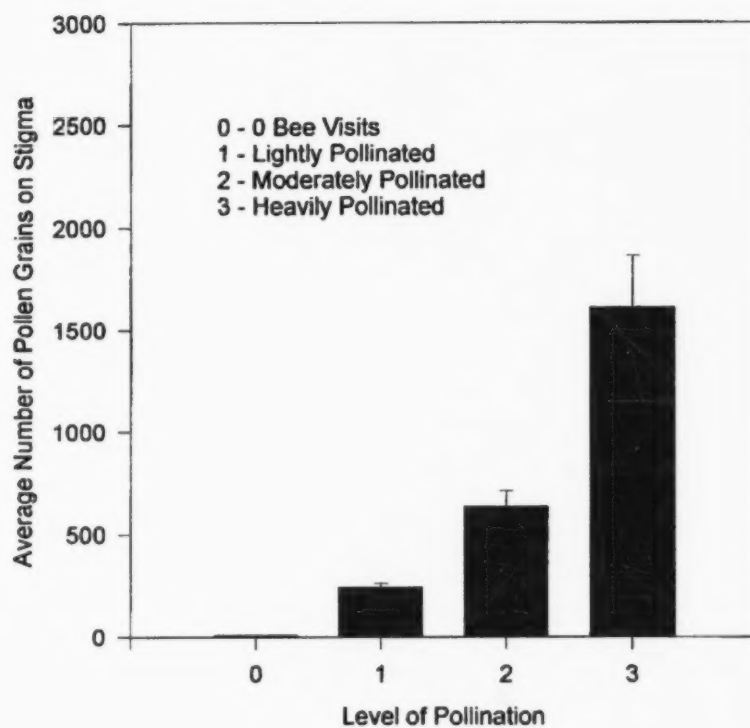


**Figure 9.** Amount of tomato pollen deposited on the stigmas of flowers of tomato plants in relation to the number of visits by bumblebees in greenhouse conditions (from Morandin 2000).

However, although greenhouses with plastics that transmit more light within the UV range promote greater bee activity and less bee loss, the growers must balance various factors when considering which kinds of plastics to install. Increased germination rates of spores of grey mold (*Botrytis*) has been associated with plastics that transmit greater amounts of UV. Thus, switching to high UV plastics becomes less desirable. Also, the temperatures in CT plastic-covered greenhouses sometimes rise above optimal for tomato growth during the summer.

#### IV. 2. High density apple orchards and pollination by honeybees

In 1999, the apple bloom (Figure 10) began on May 5, 1999. At that time only 2 cultivars had started flowering (Russett and Ida Red). Five of the remaining 12 cultivars had flowers open on May 6, and the remainder started flowering on May 7 or 8. By the last census day (May 19), all cultivars still had a small number of open flowers except for Spartan, Russett and Jonagold. Flowering curves for the six cultivars used in this study are shown in Figure 11. The cultivars surveyed fell into 5 groups defined by the day of peak flowering (Table 1). The maximum difference between Ida Red and any of the cultivars included in the across row analysis was 4 days (for Red Delicious and Northern Spy). We found significant differences in blooming times between all cultivars (Kolmogorov Smirnov test:  $p < 0.05$ ), with two exceptions; the Empire and McIntosh comparison ( $D = 0.029$ ,  $D_c = 0.037$ ,  $p = 0.21$ ) and the Spartan and Jonagold comparison ( $D = 0.046$ ,  $D_c = 0.039$ ,  $p = 0.039$ ). Measures of percentage flowering overlap with Ida Red varied from a low of 47% for Gala to a high of 87% (Table 2). Because there is overlap in phenology between cultivars it was important to determine the level of cross-compatibility between cultivars with Ida Red. We found significant differences in terms of fruit set ( $F_{4,45} = 7.51$ ,  $p = 0.0001$ ; Table 3) and total seed production (fruit set X seed production per fruit ( $F_{4,45} = 9.75$ ,  $p < 0.0001$ ; Table 3).



**Figure 10.** Apple in bloom, showing the king blossom, expanded petals, dehiscing anthers and receptive stigmata. Full pollination of the king blossoms results in the largest, most symmetrical, and highest quality apples. Pollination of the other flowers often results in smaller fruit and incomplete pollination results in incomplete seed-set and asymmetrical fruit of much lower value to growers.

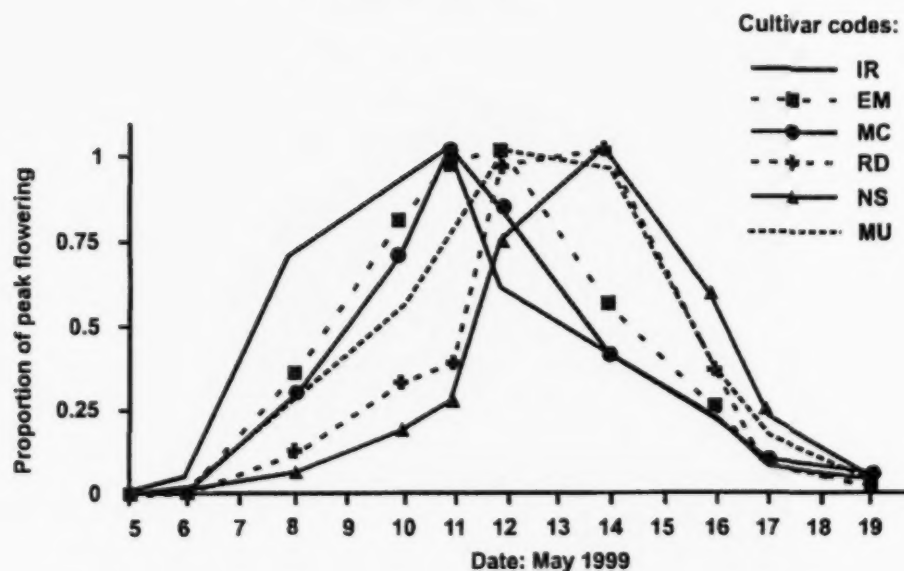


**Figure 11.** Flowering phenology for six apple cultivars at Farmer Jack's orchard near London, Ontario in 1999. IR is Ida Red; EM is Empire; MC is McIntosh; RD is Red Delicious; NS is Northern Spy; and MU is Mutsu. In 1999, like 1998, the bloom period was unusually fast.

**Table 1.** Apple cultivars in the Farmer Jack's and Dwarf Tree Orchards classified by day of peak flowering in 1999. Underlined cultivars were included in the across-row gene flow analyses.

| Group | Cultivars                                                                   | Day of Peak Flowering |
|-------|-----------------------------------------------------------------------------|-----------------------|
| 1     | Vista Bella                                                                 | May 7 to May 9        |
| 2     | Russett and Paula Red                                                       | May 9 or May 10       |
| 3     | <u>Ida Red</u> and <u>McIntosh</u>                                          | May 11                |
| 4     | Spartan, Cortland, <u>Empire</u> and <u>Mutsu</u>                           | May 12 or 13          |
| 5     | Jonagold, <u>Red Delicious</u> , Granny Smith, Gala and <u>Northern Spy</u> | May 13 to 15          |

Once we determined the physical availability of different pollen types which arises from phenological overlap and cross-compatibility, we assessed the effects of distance and apple cultivar on the percentage of seed sired by Ida Red. At Farmer Jack's, cultivar had a significant effect on the proportion of seeds sired by Ida Red ( $F_{2,21} = 6.44$ ,  $p = 0.0066$ ) but distance did not ( $F_{1,21} = 0.99$ ,  $p = 0.3308$ ). Similarly at Dwarf Tree cultivar had a significant effect on the proportion of seeds sired by Ida Red ( $F_{4,21} = 16.15$ ,  $p < 0.001$ ) but distance did not ( $F_{1,21} = 3.44$ ,  $p = 0.0779$ ).

**Table 2.** Percentage flowering overlap between 13 apple cultivars and the pollen donor cultivar, Ida Red in 1999, near London, Ontario. Underlined cultivars were included in the across-row gene flow analyses.

| Cultivar             | Percentage overlap |
|----------------------|--------------------|
| Paula Red            | 0.868              |
| Russett              | 0.86               |
| <u>McIntosh</u>      | <u>0.856</u>       |
| <u>Empire</u>        | <u>0.848</u>       |
| Vista Bella          | 0.827              |
| Cortland             | 0.806              |
| Jonagold             | 0.762              |
| <u>Mutsu</u>         | <u>0.713</u>       |
| Spartan              | 0.712              |
| Granny Smith         | 0.581              |
| <u>Red Delicious</u> | <u>0.575</u>       |
| <u>Northern Spy</u>  | <u>0.48</u>        |
| Gala                 | 0.469              |

**Table 3.** Fruit set (%) and seed / fruit for five apple cultivars after hand pollinations with Ida Red pollen ( $n$  = number of trees). Values are untransformed fruit set values. All values are means for all trees ( $n$  = 10 for fruit set and total seed; seed production based on trees with fruit). Data from 1999 near London, Ontario.

| Cultivar      | Fruit set (%)      |      | Seed set          |       | Total Seed       |       |
|---------------|--------------------|------|-------------------|-------|------------------|-------|
|               | Mean               | SE   | Mean              | SE    | Mean             | SE    |
| McIntosh      | 38.0 <sup>a</sup>  | 7.1  | 8.6 <sup>a</sup>  | 0.33  | 3.2 <sup>a</sup> | 0.62  |
| Northern Spy  | 34.6 <sup>a</sup>  | 8.1  | 12.2 <sup>d</sup> | 0.72  | 4.1 <sup>a</sup> | 0.97  |
| Mutsu         | 25.0 <sup>ab</sup> | 10.2 | 3.2 <sup>b</sup>  | 0.85  | 0.9 <sup>b</sup> | 0.43  |
| Empire        | 7.7 <sup>bc</sup>  | 3.4  | 8.6 <sup>a</sup>  | 0.63  | 0.7 <sup>b</sup> | 0.31  |
| Red Delicious | 1.3 <sup>c</sup>   | 1.3  | 7.0 <sup>c</sup>  | ----- | 0.1 <sup>b</sup> | ----- |

In Empire, Red Delicious, Northern Spy, Mutsu and MacIntosh cultivars, all seeds sired by Ida Red could be identified unambiguously. The proportion of seeds sired by Ida Red varied significantly across rows in both orchards studied ( $F_{8,18} = 31.12$ ,  $p < 0.0001$  at Farmer Jack's orchard and  $F_{8,18} = 7.74$ ,  $p = 0.0002$  at Dwarf Tree Orchard). At Farmer Jack's Orchard the mean percentage of seeds sired by Ida Red ranged from 76% in the row next to Ida Red (a Red Delicious row) to 1% in 18 rows away (also a Red Delicious row). The highest values were those closest to the Ida Red rows and 80% of all observed seeds fertilized by Ida Red were found in the first 3 rows. At Dwarf Tree Orchard, the highest percentage of seed sired by Ida Red were found 3 rows away (42%; a MacIntosh row) and the lowest 9 rows away (6%; a Mutsu row). The first 3 rows (closest to the Ida Reds) only accounted for 44% of all observed seeds fertilized by Ida Red, and the 80% level was not reached until after 9 rows.

These results indicate much variation within cultivars for male reproductive success and suggest that flowering time and distance plays an important role in determining mating patterns. Distance and flowering time are important to pollination, however, because apples are insect pollinated it is necessary to examine how bees move within orchards in order to completely understand the dynamics of pollen movement.

In 1998 on May 3 when Ida Red was in more than 50% bloom we observed bee behaviour in this cultivar. We observed that bees foraged primarily for pollen. However, the next day, and until the end of the bloom on May 9, over 80% of bees foraged for nectar and did not collect pollen. On Red Delicious and Jonagold, cultivars which reach peak bloom after Ida Red, over 90% of bees observed foraged only for nectar. Less than 15% of bees observed on any cultivar at any time during the bloom had pollen sacs of any size. Less than 20% of bees which appeared to forage mainly for pollen on Ida Red had pollen sacs.

We were able to observe 33 bees forage continuously on more than 10 flowers of Ida Red. We observed that most bees foraged either from the middle of the tree to the top of the tree (36.4%) or from the bottom of the tree to the middle of the tree (27.3%). Approximately 30% of the bees stayed only in the middle (10%), top (10%) or bottom (10%) portions of the trees. Casual observation indicated that bees tend to move along rows rather than across rows. However, unless radio trackers are used it is impossible to determine the exact movements of bees through the orchard. It is clear from the results of genetic fingerprinting that pollination from up to 18 rows away occurs. The cross-compatibility, phenological overlap and distance from pollenizer cultivar are all

factors in successful fruit set. The precise role of bees in the effectiveness of pollination remains unclear. Although bees forage primarily for nectar and do not collect pollen, their activity is sufficient to promote pollination from up to 18 rows away.

#### IV. 3. Field cucumbers and honeybee pollination

In 1998, honeybees became well distributed throughout the fields whether or not the hives were placed together, or arrayed along the field-edge in groups of two. However, there was a decline in activity so that at 250 metres, activity was half that close to the hives (ca. 10 m). The difference in honeybee activity ranged from 2.1 to 1.1 bee visits in 15 minutes. During the entire flowering season, the sex ratio of flowers was 70% male. The honeybees behaved the same way on all four cultivars (FanciPak, FanciPak-M, Vlasset and Score) examined. On the second field studied, honeybee activity was more evenly distributed, however bee activity was less than half that on the first field (Figure 12).

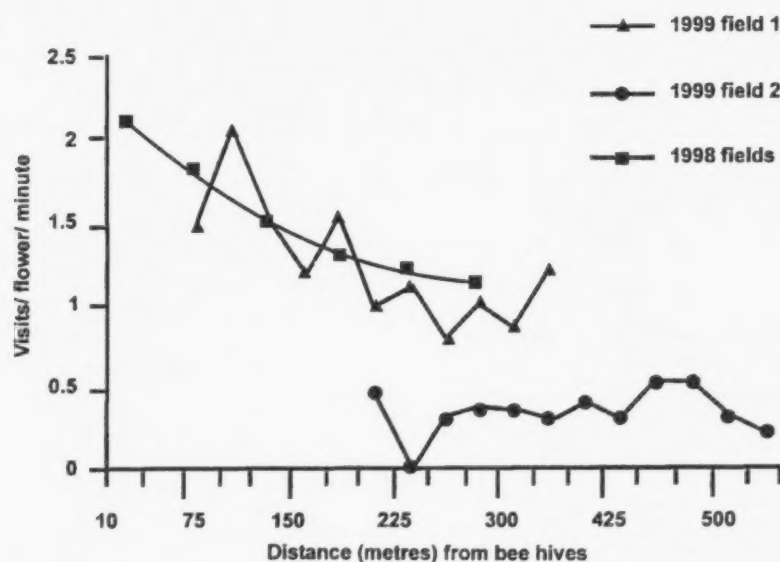


**Figure 12.** The distribution of honeybees, away from their hives at distance 0 m, across fields of blooming field cucumbers near Simcoe, Ontario. The first data point was the point at the edge of the field closest to the bee hives.

The main difference between the two fields was in hive placement relative to the cucumber fields. In the first field the hives were located so that the bees had direct access to the fields whereas in the second field the hives were separated from the cucumber fields by a pond and a row of trees. The difference in honeybee activity from an average of 2.1 to 0.25 bee visits to flowers in 15 minutes in 1998 and 1999 did not seem to be enough to be reflected in yield reductions or increasing numbers of malformed cucumbers within or across the fields.

The honey bees behaved the same way on all six cultivars (FanciPak, FanciPak-M, Vlasset, Score, Regent, Paton) examined in both years. The results of our observations on bee foraging were consistent with years for all the cultivars. Honeybee foraging activity was greatest between 12:00 and 1:00 in both years. Observations of bee behaviour with regards to choice of male or female flowers, amount of time spent on a flower, and direction of flight indicate that behaviour remained relatively constant throughout the day.

Yields in 1999 seemed comparable to those of the previous year. During the entire flowering season of 1998 the sex ratio of flowers was 70% male to 30% female. For 1999, the ratio was approximately 50% male to 50% female on both fields. The floral sex ratio influences the probability of pollination taking place, so that with the large numbers of male flowers relative to female ones, pollination of more female flowers is more assured. In neither year were the floral sex ratios as expected with 20% male flowers to 80% female flowers.

The literature survey provided a list of 15 enzymes and 26 genes previously found to be useful for distinguishing cultivars of pickling cucumber. We screened our seed collections for most of these genes. In total, all 18 cultivars were screened for 26 different enzymes (including 44 genes). Of these, 9 enzymes (12 genes) were not resolvable at all and could not be used. Fifteen enzymes (30 genes) were resolvable but showed no difference among our particular cultivars. Two enzymes (2 genes) were resolvable and showed some variation between cultivars. Those enzymes are mannose phosphate isomerase (MPI) and 6-phosphogluconate dehydrogenase (6PGD). We found that:

Picklet=Fancipak=Benson=Lafayette=PikRite;

Vlaspik=Bradley=Jackson =1925; Vlasset=Atlantis=Royal; Vlasspear=Paton;

Magic=Duke; Campbell=Sumter (Table 4).

We collected 2 different cultivars (Paton and Vlasset). For Paton, the presumed pollenizer is 1925 because that is the only pollenizer cultivar used by Sunseeds, the supplier of Paton seeds. For Vlasset the pollenizer cultivar is less clear. Asgrow supplies Vlasset seeds, but uses 3 different pollenizer cultivars (Benson, Magic and Campbell). We could not confirm with Asgrow which pollenizer cultivar was mixed in with the Vlasset seeds.

From the electrophoresis of seeds from fruit collected in the field we found that 29% of the pollen fertilizing Paton seeds has the F allele which is not present in the supposed pollenizer (1925). We also found that 29% of the pollen fertilizing Vlasset seeds has the S allele which is not present in the supposed pollenizer, if the pollenizer was one of the 2 with the "FF" genotype (Benson or Magic). If the pollenizer was the one with the SS genotype, 71% of all the pollen could not have come from the pollenizer (Campbell).

As with apples, bees are needed for pollinating pickling cucumbers. From our observations of bee activity we noted that approximately 65% of the bees moved straight down rows towards the end of the field while they visited cucumber flowers. The results of the electrophoresis suggest that the bees are not staying within the cultivars and spread pollen throughout the field. This result could have an impact on growers if the cultivar and associated pollenizer seed are selected to maximize fruit quality.

**Table 4.** Genetic signatures for 18 field cucumber varieties based on 2 enzymes. Each signature is depicted by a two letter code which refers to the specific forms of the gene present in each variety.

| Variety (listed by Seed Co.) | 6PGD | MPI |
|------------------------------|------|-----|
| <b>ASGROW</b>                |      |     |
| Picklet                      | MM   | FF  |
| Fancipak                     | MM   | FF  |
| Vlaspik                      | FF   | SS  |
| Vlasset                      | MM   | FS  |
| Vlasspear                    | FF   | FS  |
| <b>ASGROW POLLENIZERS</b>    |      |     |
| Benson                       | MM   | FF  |
| Magic                        | SS   | FF  |
| Campbell                     | SS   | SS  |
| <b>SUNSEEDS</b>              |      |     |
| Paton                        | FF   | FS  |
| Bradley                      | FF   | SS  |
| Jackson                      | FF   | SS  |
| Lafayette                    | MM   | FF  |
| <b>SUNSEED POLLENIZER</b>    |      |     |
| 1925                         | FF   | SS  |
| <b>HARRIS MORAN</b>          |      |     |
| Duke                         | SS   | FF  |
| Atlantis                     | MM   | FS  |
| Pik-Rite                     | MM   | FF  |
| Royal                        | MM   | FS  |
| <b>H. MORAN POLLENIZER</b>   |      |     |
| Sumter                       | SS   | SS  |

#### IV. 4. Pollination problems in highbush blueberry

There are significant differences in cultivars for both opening width and length of flower ( $F = 333.92$ ,  $p < 0.0001$ , and  $F = 57.61$ ,  $p < 0.0001$  respectively) (Figure 13). Patriot (not shown in Figure 13) was significantly smaller in both length and floral width than the other cultivars which were measured. Because honey bees' tongue lengths are only about 6mm long, the only cultivar which is likely to be easily pollinated by nectar gathering honeybees is Patriot.

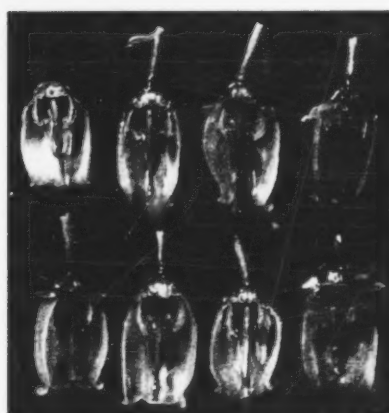


**Figure 13.** Floral forms from various cultivars of highbush blueberries commonly grown in Ontario. Flowers presented as hanging as in nature. From top-right, row one: Northland, Blue Crop, Blue Joy, Elliot; row two: Duke, Blue Ray, Jersey, Berkeley. Patriot, with the smallest flowers is not shown but has flowers less than one-half the length of those of Northland.

The depth of the flowers of that cultivar is 3.73mm, in comparison with those of Northland which has the next shallowest flowers at 8.56 mm. Although Northland's flowers are long relative to the length of a honey bee's tongue, the entrance to the flower is a relatively wide (5.14mm), thus it is possible that a honey bee could push its head into the flower far enough to gather nectar, thereby causing pollination. Duke and Berkeley are the widest flowers measured (10.34 mm and 10.46 mm respectively), however both are amongst the deepest (10.10 mm and 10.46 mm respectively) and thus are not likely to be easily pollinated by honeybees.

Two experiments which need to be conducted are 1) to determine honeybee and native bee activity on blueberry cultivars with different floral morphologies and 2) to determine the nature of "incompatibility" in the highbush blueberry cultivars grown in Ontario, controlled self and cross pollination experiments are needed. These were to consist of self-within-plant (a) versus self-within-cultivar-cross-between-plant (b) versus cross-between-cultivar (c). Unfortunately, the hot, brief springs in both 1998 and 1999 prevented these experiments from being initiated because the apple blossom and blueberry bloom overlapped.

#### IV. 5. Hoary squash bee

The hoary squash bee (*Peponapis pruinosa*) (Figure 14) is widely distributed throughout southern Ontario. Gaps in the range seem to exist in the area bordered by a line round Port Elgin, Barrie, and Listowel (Figure 15). It seems also absent north of Peterborough and onto the Shield. More in depth studies are needed to determine if there is a difference in *Peponapis* density throughout its distribution. Also studies need to be conducted to determine what habitats provide ideal nesting requirements for the bee. From our series of successful resin casts taken in summers of 1998 to 2000 of *Peponapis* nests we found that the first cell occurs at a depth of approximately 10 cm. This suggests that farmers who use conservation tillage systems would not negatively impact on nests of this native bee.

#### IV. 6. Red clover pollination

Honeybees and bumble bees had a significantly positive effect on seed set in red clover, as shown by the field yield map and results from our study plots. High seed yields corresponded to areas of high activity levels of pollinators. The growers who funded this research in 1999 have requested that there be a temporary hold on the release of detailed results. The OREP budget was not sufficient to carry out this research without the major input of grower funds.



**Figure 14.** An adult female of the hoary squash bee, *Peponapis pruinosa* enters her underground nest near Ancaster, Ontario.

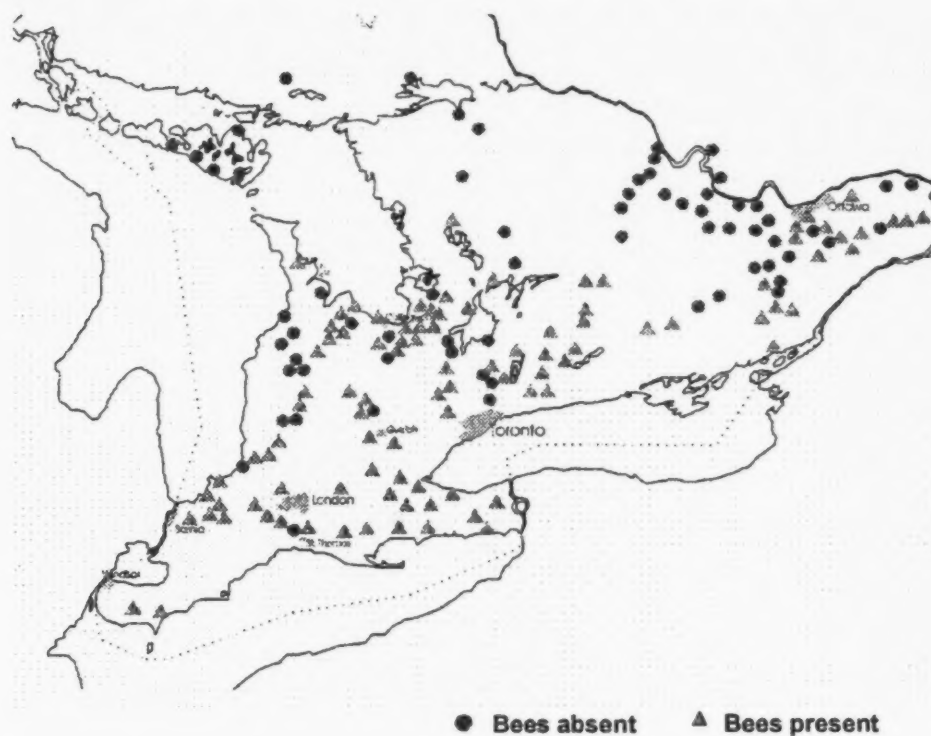


**Figure 15.** The distribution of the hoary squash bee, *Peponapis pruinosa*, in Ontario from surveys of 1998 and 1999.

## **V. Conclusions**

### **V. 1. Greenhouse tomatoes and pollination by bumblebees**

CT plastic is the best plastic tested in terms of bumble bee activity and for reduction of bee loss. The greater transmission of UV wavelengths and higher temperature are problematic for tomato production.

### **V. 2. High density apple orchard and pollination by honeybees**

Genetic fingerprinting provides a clear picture of gene flow patterns through high density apple orchards. Pollen donors are typically within 3 rows away; however a successful donor can be up to 18 rows away from the maternal tree. This suggests that the chosen pollenizer cultivar should be in close vicinity of the maternal trees. Distance, as well as flowering overlap play important roles in the success of a pollenizing donor. Although honeybee pollination is important, it is difficult to determine the consequences of honeybee pollination on any one particular apple cultivar merely with observations of bee activity.

### **V. 3. Field cucumbers and honeybee pollination**

Because honeybees work over an entire field, and do not remain true to any one cultivar and its associated pollenizer, there is the potential for gene flow throughout the field. This might have implications for the grower if the pollenizer was specifically selected to maximize cucumber production from a given cultivar.

### **V. 4. Pollination problems in highbush blueberries**

The differences in floral structure, size and shape between blueberry cultivars may be important for successful pollination by honeybees.

### **V. 5. Hoary squash bee**

This native bee occurs throughout southern Ontario. From our 7 successful resin casts of *Peponapis* nests we found that the first cell occurs at a depth of approximately 15 cm. This suggests that farmers who use conservation tillage systems would not negatively impact on nests of this native bee.

#### **V. 6. Red clover pollination**

Our preliminary study has indicated that honeybees are more effective than bumble bees or native bees in the pollination of red clover. The much higher density of honeybees (than of bumblebees) foraging from the strong colonies we used may account for our results.

## VI. Future Directions

1. The cost effectiveness of using CT plastic versus other greenhouse plastics must be determined. The economic gains provided by decreased loss of bumble bees through vents in the winter and coupled with greater activity under CT plastics must be balanced against the economic losses caused by grey mold and lower tomato production as the result of greater UV light transmission and less than optimal greenhouse temperatures.
2. Further genetic fingerprinting of additional apple cultivars is required to get a better handle on gene flow patterns in different cultivars within high density apple orchards.  
Although bee observations provided basic information regarding pollinator movements, further work on the exact number of visits which are required per flower to maximize apple quality, and the effect that distance from the hive has on bee visits may provide more useful information to the growers.
3. Observations of pollinator activity on different blueberry cultivars would be useful for understanding the effects of the different floral morphologies on pollinator activities. There is much to learn about what pollinates blueberries, how reliably, the habitat requirements for the best pollinators, encouragement to nest near the fields, and so on. In various places, specialist pollinators have been advocated and encouraged for various blueberry crops (see Kevan *et al.* 1990), an approach which would serve well in Ontario.
4. Further work is needed on gene flow through cucumber fields and the implications of cross cultivar/pollenizer pollinations.
5. More in depth studies are needed to determine if there is a difference in *Peponapis* density throughout its distribution. Also studies need to be conducted to determine what habitats provide ideal nesting requirements for the bee.
6. The red clover experiment needs to be replicated on more fields to confirm our results that honey bees are more effective than bumble bees and native bees.

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## VIII. Partnerships

During this project partnerships were formed with grower organizations as follows:

- Ontario Apple Marketing Commission
- Ontario Vegetable Growers' Marketing Board
- Ontario Fruit and Vegetable Growers' Association
- Bick's Pickles/Multifoods Inc.
- Ontario Beekeepers' Association

Those partnerships have allowed for further funding from 1999 - 2001 to be obtained from The Natural Sciences and Engineering Research Council of Canada, Agriculture and Agri-Food Canada, and Ontario Ministry of Agriculture, Food, and Rural Affairs

## Appendix I

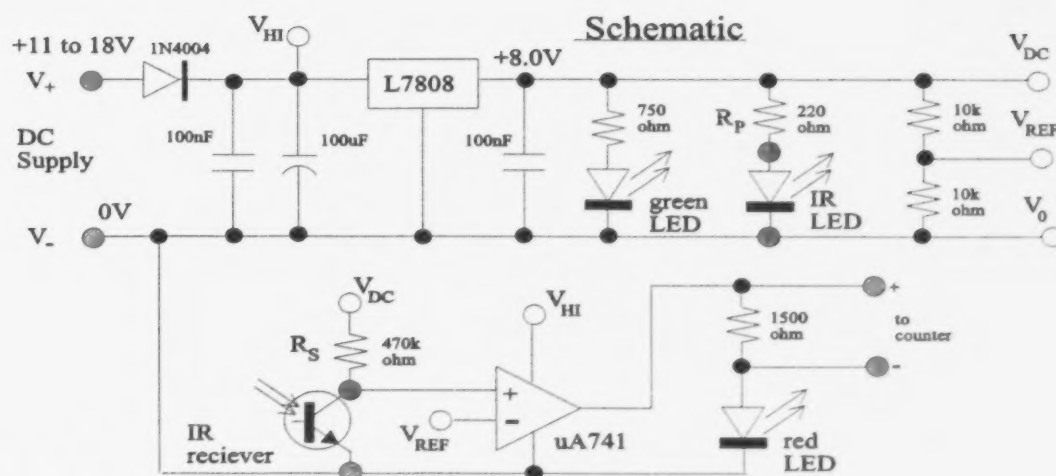
### MEASURING FORAGING ACTIVITY IN BUMBLE BEE NESTS: A SIMPLE HIVE ENTRANCE TRIP COUNTER

designed and built by  
Eric Praetzel, University of Waterloo, Waterloo, Ontario

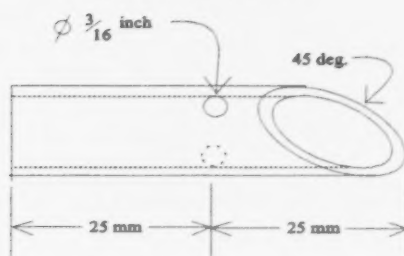
Bumblebees have become increasingly important as commercially available pollinators, especially in the glass house industry and for tomato production. Although these bees work flowers of tomatoes efficiently and bring about levels of pollination that exceed that eventuating from pollination by hand-held buzzers, there are various questions that relate to the bees' activity levels and pollination efficiencies in glass houses. For example, the nature of the coverings of the houses influences activity, as do regimes of temperature, humidity, and light (Morandin 2000). To assess the activities of bumblebees under glass house conditions, we developed a demountable counter that can be easily fitted to the entrance of commercially available bumblebee hives. The device we have developed can also be used on bumblebee hives outdoors, and could, no doubt be adapted to other applications.

Our device uses an infrared (IR) beam to detect the passage of bees through a tunnel, which is attached to the entrance into the bee hive. The IR beam operates at a wavelength of 950nm so is invisible to bees. The basic operation of the sensor is straight forward.

An unregulated DC power source is needed taken from the AC mains electrical supply though an AC/DC adapter that filters and regulates the power. An IR emitter is mounted on top of the entrance tunnel and the IR receiver on the bottom is used to monitor the beam of light. If the IR beam is obstructed by a bee then a voltage comparator senses this change and signals the counter to register an increment of one.



### Sensor Head

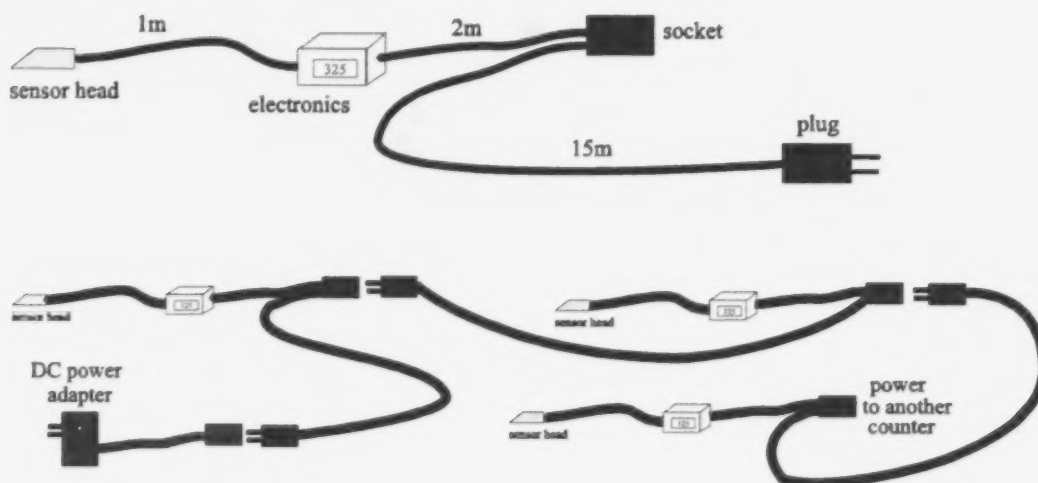


### Legend

- External connection
- IR -Infrared

### The Physical unit

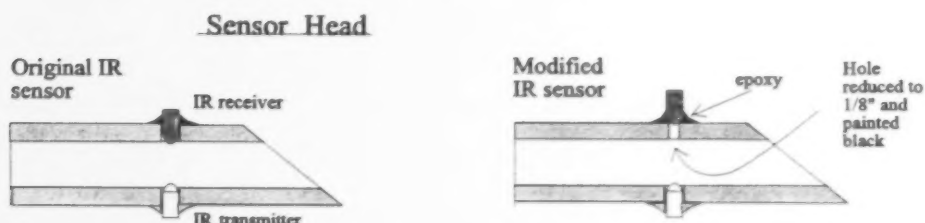
Each bee counter unit (box and electronics) has an external sensor head holding the IR beam electronics in addition to a power cable with a socket and a plug. The plug connects to the DC power adapter or to the socket of another power cable that eventually leads to a DC power adapter. A single DC power adapter can power up to 5 bee counter units. Physically a unit looks like this singly or when several units connected together:



One of the concerns of using such a counter is its accuracy. Although the counter counts exactly and only the number of times the light beam is interrupted. It is known that bumblebees, as other bees, may venture to the exit of the nest only to turn round and return to the interior. It is also well known that colonially nesting bees may post guards at the nest entrances. Both behaviours would result in counts additional to those reflecting foraging trips. Our counters were designed so that only one bee at a time could easily walk through the tube. Thus, the counts we obtained were of individual bees breaking the light beam. To test the accuracy of the counters we watched bumble bees actually leaving and entering colonies in glass houses for standard periods (details) and then compared the actual foraging counts with the counts obtained by the counter. Our results showed that the counter values and actually observed entrances and exits are highly correlated ( $r^2 = 0.85$ ;  $F_{24} = 129.75$ ;  $p < 0.0001$ ), with about 3 counts per actual entrance or exit.

### Construction Details

- 1) The sensor head plugs into the beehive entrance and holds the IR emitter and receiver. The inside of the sensor head must be painted black (Tremclad flat black) to reduce light reflections from the sky and sun hitting the IR receiver. The IR receiver and transmitter must also be painted black, on the outside, to prevent them from passing IR light into the sensor head.
- 2) The IR receiver and transmitter wires must be fixed into place with something like RTV silicone to prevent movement and breakage. The wire bundle (all 4 sensor wires) must be tied to the sensor head with a cable tie to prevent movement.



- 3) For improved operation in direct sunlight the IR receiver has to be recessed to prevent stray light from hitting it. To prevent stray light and reflections the hole is reduced to 1/8" and painted black. The IR transmitter power had to be increased by decreasing the resistance of  $R_p$ . The IR receiver sensitivity may improved by increasing the resistance of  $R_s$ . The size of these changes depends upon the type of black paint used and the diameter of the IR receiver hole.

### Parts List

1 part per unit unless otherwise stated.

All prices are per 100 quantity unless otherwise stated.

Plastic Box: Hammond 1591BSBK (4.4" \* 2.5" \* 1.06") \$1.55

Counter: Hengstler GO-731-101, \$63.40

Note: Cheaper counters are available but many do not have a reset.

Resistors: 5% tolerance carbon, \$2.00

220 ohm, 750 ohm, 1.5k,

2 of 10k, 470k

Capacitor: 2 of 100nF ceramic i.e. Phillips CZ20C104M, \$9.08

Capacitor: 25V, 100uF electrolytic, \$38

Opamp: Sayal, UA741CN, \$60

Voltage Regulator, Sayal, L7808, 8V, 1A, \$75

Infrared receiver: Sayal 58995, \$60

Infrared transmitter: Sayal 58994, 950nm, 100mA, \$60

LEDs: 1 each of green and red standard 5mm

Diode: 1N4004, \$4.20

Circuit Board: 2" \* 1.5" per unit, Vector 45P80-1 (3" \* 8"), \$38.40 each in quantities of 10

DC Power adapter: one adapter per 5 units, Sayal 12Vdc, 500mA, \$7.90 each

power cable connectors: 1 plug and one socket per unit, one socket per DC Power adapter

Cinch plug P-302-CCT, \$2.80

Cinch socket S-302-CCT, \$2.86

power cable wire: used 50' per unit, Beldon unreel, 20ga, 2 conductor,

8205-U1000-60, \$126 per 1000 foot reel

sensor wire: used 2' per unit, Beldon unreel, 22ga, 4 conductor, unshielded, \$0.20 per foot

Beehive sensor head body: Canadian Tire, CPVC 1/2" white tubing

paint: Tremclad flat black

epoxy: 24 hour (to mount IR parts to CPVC tubing)

clear RTV 100% silicone: Sayal (used to glue counter to plastic box and glue wires)

Velcro: 1.5" per unit, Sayal, hook, adhesive back 25mm wide, \$12 for 10'

Velcro: 1.5" per unit, Sayal, loop, adhesive back 25mm wide, \$12 for 10'

Plastic cable ties: 2 per unit, Tyraps std. 4\*2.5mm, \$5.00

### Suppliers

Electrosonic Inc, 1-800-56-SONIC, FAX 416-496-3030,  
1100 Gordon Baker Rd, Willowdale, Ontario M2H 3B3

Sayal, [www.sayal.com](http://www.sayal.com), 416-494-8999 FAX 494-9721

3791 Victoria Park Ave, Unit 1-4 Scarborough, Toronto, Ontario M1W 3K6



